Mucorales isolated from pine root bark and wood

E. G. KUHLMAN
Southeastern Forest Experiment Station, United States Department of Agriculture, Research Triangle Park, North Carolina
Received May 8, 1969

KUHLMAN, E. G. 1969. Mucorales isolated from pine root bark and wood. Can. J. Bot. **47**: 1719–1723.

Thirty species of Mucorales were recovered in isolations of organisms associated with *Fomes annosus* and other wood-rotting fungi in decay of *Pinus taeda* and *P. elliottii* var. *elliottii* root bark and wood. Fifteen to 20% of the isolates from roots in advanced decay were Mucorales. *Mortierella* spp. were most abundant; some of them are described.

Introduction

This report is part of a study to determine the organisms associated with *Fomes annosus* (Fr.) Karst. and other wood-rotting fungi in decay of southern pine root bark and wood. Most studies of microbial interaction have devoted little attention to Mucorales. However, species of Mucorales are common constituents of the soil microflora (3, 13, 14, 15, 23) and some are considered "sugar fungi" (4). The abundant distribution of "sugar fungi" throughout the soil and on organic debris in the soil results from their rapid growth and prolific spore production wherever simple carbon compounds are available (4). The saprophytic ability of root-infecting fungi depends, in part, on their capacity to compete with the "sugar fungi" for simple substrates.

This paper lists the species of Mucorales that I isolated from bark and from wood in incipient and advanced stages of decay. Literature on the taxonomy of the Mortierellaceae is discussed, and descriptions are presented for some species of *Mortierella* that were isolated.

Materials and Methods

Members of the Mucorales were isolated from bark and wood of roots in three ages or stages of decomposition. The wood was free from decay, or in an incipient stage of decay, in root pieces of loblolly pine (*Pinus taeda* L.) buried in a pine stand in Research Triangle Park, North Carolina, and in stump roots of slash pine (*P. elliottii* Engelm. var. *elliottii*) on the Sand Hills State Forest, South Carolina (11). Wood of loblolly pine roots was in an advanced stage of decay in studies on the Sand Hills State Forest (10). Dead loblolly pine seedlings near Summerville, S.C., were also a source of isolates.

Roots, root pieces, or seedlings were removed from the soil, cleansed in water, and dipped in 65% ethyl alcohol. Cultures were made by removing chips of wood or bark from sample points, generally at 2.5-cm intervals, and placing the chips on either malt-extract agar (MEA) or a previously described (12) medium selective for *F. annosus* (FAM).

For identification, the isolates belonging to the Mucoraceae were grown on MEA. The Mortierellaceae were grown on Thornton's (20) standardized medium (TSM), MEA, and soil extract agar (SEA). All cultures were incubated under room conditions at 20–25 °C for 1–2 weeks.

Results and Discussion

Isolation

Thirty species representing seven genera of Mucorales were isolated (Table I). Fifteen species of *Mortierella* were recognized, including a sterile form that resembled *Mortierella* in growth habit, presence of oil droplets, and characteristic garlic odor. *Mortierella* spp. were isolated with much greater frequency than were other Mucorales. *Mortierella bainieri*, *M. candelabrum*, *M. isabellina*, *M. marburgensis*, *M. nana*, *M. pulchella*, and *M. ramanniana* were the most commonly isolated species. *Mortierella nana* was a common component of the mycoflora of wood in advanced stages of decay but was not present in incipient decay.

Isolations from the roots in advanced decay indicated the Mucorales were equally abundant in bark and wood. Of more than 1200 isolates recovered, 15% of those from root wood and 19% from root bark were Mucorales. Preliminary evidence from in vitro studies suggests the Mucorales depend on breakdown products from other organisms for their growth. Because fewer isolations were made from roots in initial than in advanced stages of decay, the relative occurrence of the Mucorales in bark and wood could not be determined.

Linnemann (13) also reported that *M. isabellina*, *M. jenkini*, *M. pulchella*, and *M. zychae* occurred in rotten wood. Sewell (18) isolated *Absidia orchidis* (= *A. coerulea*), *M. isabellina*, *M. marburgensis*, *M. zychae*, *Mucor ramannianus* Möll. (= *Mortierella ramanniana* (Möll.)

Linnemann), and *Mucor hiemalis* from the surface of *Calluna* roots after repeated washings. Chesters (1) isolated a number of *Mortierella* spp. from root debris, but did not identify them. Meredith (16) indicated that the number of Phycomycetes, especially *Mucor ramannianus* and *Mucor* spp., increased with age of pine stumps. Similarly Kaarik and Rennerfelt (9) found *M. ramannianus*, *Mucor* spp., and sterile Phycomycetes on 4-year-old and older pine and spruce stumps.

Previously Kuhlman and Hendrix (12) reported no recovery of Mucorales on FAM because of probable inhibition by pentachloronitrobenzene (PCNB). This failure may have occurred because they were isolating from aboveground portions of the stump rather than from roots. Mucorales generally grow less luxuriantly on FAM than on MEA. In current studies, a greater variety of Mucorales was isolated on MEA than on FAM; however, many species of *Mucor* and *Mortierella* were isolated on both media.

TABLE I

Species of Mucorales isolated from bark and wood

Name of fungus	Bark	Wood
	Sources*	
Absidia coerulea Bainier	2	2
Cuninghamella elegans Lendn.	2	2
Gongronella butleri (Lendn.) Peyronel & Dal Vesco	2	2
Mortierella bainieri Constantin	1, 2	2
M. candelabrum Van Tiegh. & LeM.	1	1, 2, 3
M. elongata Linnemann	2	2
M. isabellina Oudemans	2	2
M. jenkini (Smith) Naumov	2	2
M. lignicola (Martin) Gams & Moreau		2
M. marburgensis Linnemann	1, 2	2
M. minutimissima Van Tiegh.	1, 2	1, 2
M. nana Linnemann	2	2
M. pulchella Linnemann	1, 2	1, 2
M. ramanniana (Möll.) Linnemann	1, 2	2
M. verticillata Linnemann	2	
M. vinacea Dixon-Stewart	2	2
M. zychae Linnemann	2	2, 3
Mortierella spp.	1, 2	
Mucor attenuatus Linnemann	2	2
M. fragilis Bainier	2	
M. hiemalis Wehmer	2	2
M. lamprosporus Lendn.		2
M. racemosus Fresenius	2	2
M. silvaticus Hagem		2
M. spinescens Lendn.		2
M. subtilissimus Oudemans	2	2
M. varians Povah	2	2
Mucor spp.	2	2
Rhizopus nigricans Ehrenberg		2
Zygorrhynchus moelleri Vuill.	2	2

*Key to sources: 1 = loblolly roots and slash pine roots having no decay or incipient decay from North and South Carolina; 2 = loblolly pine roots in advanced decay from South Carolina; 3 = dead loblolly pine seedlings from South Carolina.

Culture

Identification of species of *Mortierella* was greatly facilitated by growing them on TSM. The amount of sterile aerial mycelium is greatly reduced, and the fertile mycelium is usually abundant. Complex branching habits, such as those of *M. candelabrum* and *M. bainieri*, are readily seen since the sporangiophores arise near the agar surface relatively free from aerial mycelium. Soil extract agar has been recommended for cultural identification of *Mortierella* (3, 22); however, in this study fruiting was more abundant on TSM than on SEA. In addition, TSM has the advantage of being a chemically defined medium; Harris and Keeney (6) have demonstrated that different soils and extraction techniques cause considerable variation in soil extracts.

Cultural Characters

Species of Mucoraceae were readily identified from keys by Gilman (5) and Ellis and Hesseltine (2, 8). Because members of the Mortierellaceae have been less frequently encountered than species of Mucoraceae, they have not been well described. Detailed descriptions of some *Mortierella* species that were isolated are given here to help establish their characteristics. Except where noted all observations were made on TSM.

There is some taxonomic confusion in the genus *Mortierella*. Hesseltine (7) separated the genus *Haplosporangium* Thaxter from *Mortierella* on the basis of sporangia with one or two sporangiospores versus those with few to many spores. Linnemann (13), however, in her monograph on the genus *Mortierella*, included many single-spored species in the genus. She considered septation in sporangiophore-bearing hyphae, together with tapered sporangiophores, as being more important than the number of sporangiospores as a basis for identifying the genus *Haplosporangium*. Subsequently Gams

and Moreau (3) transferred three species of *Haplosporangium* to *Mortierella* and suggested that the other known species of *Haplosporangium* might also be included in *Mortierella.*

The *Mortierella* spp. are usually separated on the basis of cottony versus velvety growth habits (3, 13, 21). This major separation is readily made on MEA. Descriptions of some *Mortierella* species follow.

M. bainieri (Fig. 1)

Sporangiophores sympodially branched with as many as six branches, usually with one axis, although at times producing two or three main branches 250–450 μ high, 10–15 μ wide at base tapering to 3–6 μ at tip. Sporangia containing more than 20 spores. Spores predominantly cylindrical although some are oval, 4–6 × 7–10 μ, thin walled. Gemmae (chlamydospores) present, 15–20 μ intercalary or terminal.

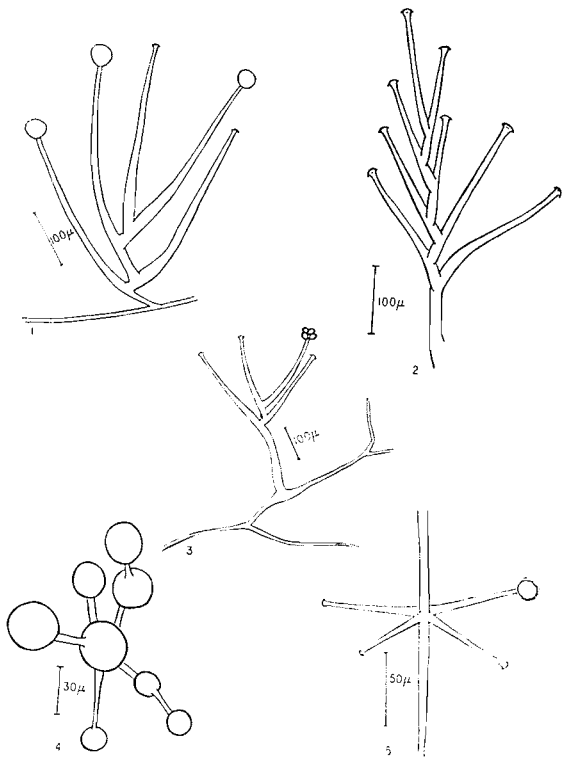

FIG. 1. Sympodial branching habit of *M. bainieri.* FIG. 2. Sympodial branching habit of *M. candelabrum.* FIG. 3. Sympodial branching of *M. candelabrum.* FIG. 4. Clump of chlamydospores of *M. zychae* on TSM. FIG. 5. Whorl of sporangiophores on aerial hyphae in *M. marburgensis.*

M. candelabrum (Figs. 2, 3)

Sporangiophores sympodially branched as in *M. bainieri*, up to 500 μ high, 10–15 μ wide at base, 3–7 μ at tip. Sporangia containing about 40–60 spores. Spores thick walled, irregularly oval to globose 5–8 × 6–12 μ, mostly 6–8 μ. Gemmae, 10–15 μ in diameter, both terminal and intercalary.

No type specimen of this species is available for comparison. Both Linnemann (13) and Zycha (24) described the branching habit of *M. candelabrum* as sympodial. The sympodial branching habit of *M. candelabrum* is like that in *M. hygrophila*; however, *M. hygrophila* has few-spored sporangia with only 5–15 irregularly shaped spores which may be up to 25 μ long. In addition, the spores in NRRL isolate A 7608 of *M. hygrophila* germinate *in situ*; those in *M. candelabrum* do not.

Linnemann (13) placed *M. candelabrum* with species that had stylospores (stalked chlamydospores), although she had not seen a specimen of the fungus and was dubious about reports that it had stylospores. Zycha (24) reported that it did not have stylospores. My isolates did not produce stylospores.

M. zychae

Sporangiophore branched as in *M. bainieri* although up to 1 mm high, 7–15 μ wide at base, 3–7 μ at tip. Sporangia many-spored (40–60). Spores globose to elliptical, 5–7 × 6–10 μ, predominantly thin walled. Gemmae produced in large clumps especially in SEA (Fig. 4).

M. marburgensis

The sporangiophores arise in irregular whorls at frequent intervals along the aerial mycelium (Fig. 5). The larger sporangiophores may have secondary branches (Fig. 6). Sporangiophores are 25–100 μ high (Linnemann (13) described them 30–180 μ), 2–3 μ wide at base to 0.5–1 μ at tip. Sporangia are 1- to 10-spored, 8–12 μ in diameter. Spores globose to oval, 4–10 μ, with thick, often roughened walls. Gemmae absent.

This species is transitional between the single-spored and the polyspored species. When first isolated the spore number varies from 1 to 10; with repeated transfers it becomes predominantly single-spored. When combining *M. marburgensis* with *Haplosporangium fasiculatum*, Gams and Moreau (3) did not mention that the spores are

thick walled and often appear roughened, as described by Nicot (17) rather than thin walled as originally described by Linnemann (13).

M. nana

Like *M. marburgensis* the sporangiophores arise at frequent intervals along the aerial mycelium. Often, however, only one sporangiophore arises at each point. The sporangiophores are 15–95 μ long, 1–2 μ wide at base to 1 μ at tip, with occasional short branches. Sporangia have a single globose spore 4–6 μ in diameter. Gemmae absent.

Turner (21) presents excellent illustrations of this species. Isolates are slow growing and usually grow less than 60 mm in diameter in 2 weeks. The gross appearance on MEA is cottony, but unlike other members of this genus it forms no rosette patterns.

M. lignicola

Stylophores short, 35–50 μ long, 3–7 μ wide at base to 1–2 μ at tip. The irregular branching habit frequently looks like fingers extending from a hand (Fig. 7). The tip of the stylophore comes to a point. The stylospores are 8–12 μ in diameter and have short, distinct spines.

Gams and Moreau (3) considered *M. sepedonioides* Linnemann and *Haplosporangium lignicola* Martin synonymous after examining stock cultures. The stylospore, as represented in this species, presents another transition within this diverse genus. Although some workers (17, 19) have considered the stylospore as being like a single-spored sporangium, it does not leave a collarette typical of the sporangia of so many other species of *Mortierella* (3). However, the thick-walled sporangiospores of *M. hygrophila* and *M. marburgensis* are suggestive of stylospores. Because there are species with both stylospores and sporangia, it would not seem logical to remove from the genus the group having only stylospores.

M. verticillata

Stylophores are 50–150 μ long, 1–3 μ wide at base to 0.5–1 μ at tip, with verticillate whorls of secondary branches. The stylospores are round (9–16 μ) with a spiny surface.

The stylospores of *M. lignicola* are the same size as those of *M. verticillata*, but the former has shorter, stouter stylophores.

M. pulchella

Sporangiophores mostly 75–125 μ, occasionally to 270 μ high, 5 μ wide at base to 1–2 μ at tip. Unbranched, or one to three side branches in whorls; side branches generally do not overreach the main branch (Figs. 8, 9). Less frequently the side branch is rebranched. Sporangia, 10–18 μ, with more than 20 spores. Spores 2–3 μ globose, to 4 × 5 μ oval, thin walled. Gemmae absent.

Linnemann (13) described this species and stated that her isolates also had abundant root-like rhizoids. My isolates had no rhizoids, but they agree with her description in other respects. Turner and Pugh (22) described two isolates similar to mine. They provisionally called their isolates *M. turficola* Ling-Young. The branches on their isolates arose midway on the main axis, but on my isolates the branches arise two-thirds to three-fourths up the main axis. Furthermore, Linnemann's description of *M. turficola* indicates the primary branching is sympodial; my isolates have monopodial whorls.

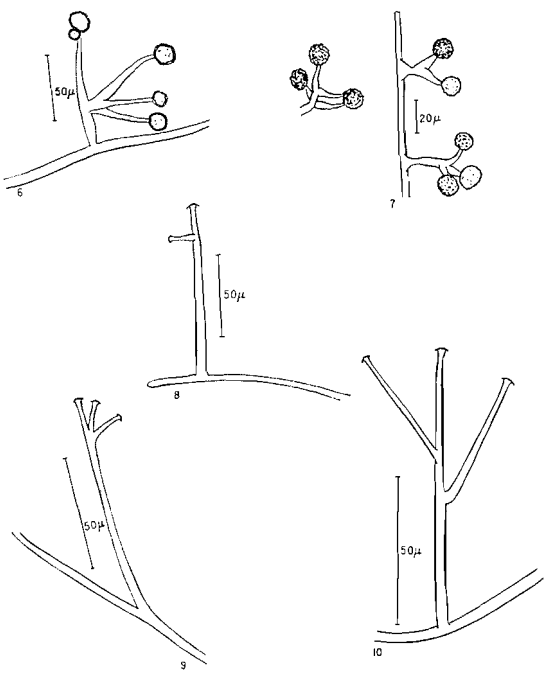

FIG. 6. Branched sporangiophores of *M. marburgensis*. FIG. 7. Irregular branching habit in *M. lignicola*. FIG. 8. Simple branching habit of *M. pulchella*. FIG. 9. Secondary branches below the primary sporangiophore in *M. pulchella*. FIG. 10. Cymose branching habit of *M. minutimissima*.

M. minutimissima

Sporangiophore 50–100 μ high, 3–5 μ wide at base to 1–3 μ at tip, unbranched or one to two cymosely arranged side branches (Fig. 10). Sporangia, 10–15 μ, with 10–20 spores. Spores, globose to oval, 5–10 μ, mostly 5–7 μ, thin walled. Gemmae absent.

This species is well illustrated by Linnemann (13) and by Turner and Pugh (22).

M. elongata

Sporangiophores up to 450 μ, little or sympodially branched. Sporangia globose, 20–25 μ. Spores irregular, mostly cylindrical 5 × 10–15 μ, some oval 3–8 × 5–10 μ. Gemmae intercalary and terminal, globose, 10–18 μ.

Linnemann (13) reported one strain with up to six branches and believed this was transitional to *M. hygrophila.* Cylindrical spores are also predominant in *M. bainieri*; however, the largest spores are bigger and there is more variation in size in *M. elongata* than in spores of *M. bainieri.*

M. isabellina and Related Species

Turner (21) has adequately described these species. *M. ramanniana* presents the only difficulty because it is a transitional species between *Mucor* and *Mortierella.* Linnemann (13) placed it in *Mortierella* because its columella was small and it resembled members of her section *Pusilla* in growth habit. Zycha (24) considered it transitional but placed it in *Mucor.* Recently Turner (21) gave reasons similar to Linnemann's for placing it in the genus *Mortierella.*

Acknowledgments

The support and assistance of Dr. C. S. Hodges is gratefully acknowledged. Mrs. H. P. Tillerson prepared the illustrations.

1. Chesters, C. G. C. 1949. Concerning fungi inhabiting soil. Trans. Brit. Mycol. Soc. **32**: 197–216.
2. Ellis, J. J. and Hesseltine, C. W. 1965. The genus *Absidia*: Globose-spored species. Mycologia, **57**: 222–235.
3. Gams, W. and Moreau, R. 1959. Le Genre *Mortierella* Coemans. Ann. Sci. Univ. Besançon Med. **3**: 95–105.
4. Garrett, S. D. 1956. Biology of root-infecting fungi. University Press, Cambridge.
5. Gilman, J. C. 1957. A manual of soil fungi. The Iowa State College Press, Ames.
6. Harris, R. F. and Keeney, D. R. 1968. Towards standardization of soil extracts for microbial media. Can. J. Microbiol. **14**: 653–659.
7. Hesseltine, C. W. 1955. Genera of Mucorales with notes on their synonymy. Mycologia, **47**: 344–363.
8. Hesseltine, C. W. and Ellis, J. J. 1964. The genus *Absidia*: *Gongronella* and cylindrical-spored species of *Absidia.* Mycologia, **56**: 568–601.
9. Kaarik, A. and Rennerfelt, E. 1957. Investigations on the fungal flora of spruce and pine stumps. Medd. Skogsforsk. Inst. **47**: 1–88.
10. Kuhlman, E. G. 1968. Survival of *Fomes annosus* in the presence of competing fungi in loblolly pine roots. Phytopathology, **58**: 729.
11. Kuhlman, E. G. 1969. Number of conidia necessary for stump root infection by *Fomes annosus.* Phytopathology, **59**: 1168–1169.
12. Kuhlman, E. G. and Hendrix, F. F., Jr. 1962. A selective medium for the isolation of *Fomes annosus.* Phytopathology, **52**: 1310–1312.
13. Linnemann, G. 1941. Die Mucorineen-Gattung *Mortierella* Coemans. Pflanzenforschung H. **23**: 1–64.
14. Linnemann, G. 1953. Einige neue erdbeushnende *Mortierella-arten.* Zbl. Bakt. II. Abt. **107**: 225–230.
15. Linnemann, G. 1958. Untersuchungen zur Verbreitung und Systematik der Mortierellen. Arch. Mikrobiol. **30**: 256–267.
16. Meredith, D. S. 1960. Further observations of fungi inhabiting pine stumps. Ann. Bot. (London) N.S. **24**: 63–78.
17. Nicot, J. 1957. Deux Mucorales die sol: additions au genre *Haplosporangium* Thaxter. Bull. Trim. Soc. Mycol. Fr. **73**: 88–93.
18. Sewell, G. W. F. 1959. Studies of fungi in a Callunaheathland soil. I. Vertical distribution in soil and on root surfaces. Trans. Brit. Mycol. Soc. **42**: 343–353.
19. Thaxter, R. 1914. New or peculiar Zygomycetes. III. *Blakeslea, Dissophora, Haplosporangium.* Bot. Gaz. **58**: 353–366.
20. Thornton, H. G. 1922. On the development of a standardized agar medium for counting soil bacteria with especial regard to the repression of spreading colonies. Ann. Appl. Biol. **9**: 241–274.
21. Turner, M. 1963. Studies in the genus *Mortierella.* I. *Mortierella isabellina* and related species. Trans. Brit. Mycol. Soc. **40**: 262–272.
22. Turner, M. and Pugh, G. J. F. 1961. Species of *Mortierella* from a salt marsh. Trans. Brit. Mycol. Soc. **44**: 243–252.
23. Warcup, J. H. 1951. The ecology of soil fungi. Trans. Brit. Mycol. Soc. **34**: 376–399.
24. Zycha, H. 1935. Mucorineae. Kryptogammenflora d. Mark Brandenburg **6***a*: 1–264.